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PATENT
Docket No.: **19603/468 (CRF D-1595C)**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Barany et al.	Examiner: J. Ricigliano
Serial No.	:	08/794,851	
Filed	:	February 4, 1997	Art Unit: 1627
For	:	DETECTION OF NUCLEIC ACID SEQUENCE DIFFERENCES USING THE LIGASE DETECTION REACTION WITH ADDRESSABLE ARRAYS	

#31
SUGGS
2-6-01

REQUEST FOR RECONSIDERATION

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In response to the August 3, 2000, office action, applicants respectfully request reconsideration.

The July 21, 2000, personal interview between Examiner Ricigliano, applicant Francis Barany, and applicants' undersigned attorney is gratefully acknowledged. The substance of that interview is summarized *supra*.

The rejection of claims 1-5, 11-21, 24-43, 45-66, 75-77, 79-80, 83, 87-88, and 138-151 under 35 U.S.C. § 103 for obviousness over Wiedmann, et. al., "Ligase Chain Reaction (LCR)—Overview and Applications," PCR Methods and Applications pp. S51-S64 (1994) ("Wiedmann") in view of Barany, "The Ligase Chain Reaction in a PCR World," PCR Methods and Applications pp. 5-16 (1991)("Barany PCR"), U.S. Patent No. 5,415,839 to Zaun et. al. ("Zaun"), Guo, et. al, "Direct Fluorescence Analysis of Genetic Polymorphisms by Hybridization with Oligonucleotide Arrays on Glass Supports," Nucl. Acids Res. 22(24): 5456-65 (1994)("Guo"), and U.S. Patent No. 5,648,213 to Reddy et al. ("Reddy") is respectfully traversed.

Wiedmann describes the use of the ligase chain reaction ("LCR") procedure to detect single base differences in target nucleic acids. LCR is disclosed to involve the use of 2

pairs of oligonucleotide probes. The first pair of probes is configured to hybridize to a first nucleic acid strand, while the second pair of probes is designed to hybridize to a second nucleic acid strand complementary to the first nucleic acid strand. The first and second pair of oligonucleotide probes are complementary to one another. As a result of using both pairs of oligonucleotide probes targeted to complementary nucleic acid strands, the LCR procedure is able to achieve exponential amplification. Wiedmann distinguishes the LCR procedure from the ligase detection reaction ("LDR") technique by virtue of the fact that LDR only uses a single pair of oligonucleotide probes which will hybridize to only one target nucleic acid strand and, thus, achieve linear amplification. There is no suggestion in this reference of detecting ligated products of an LDR procedure using a solid support, having capture probes which hybridize to an addressable array-specific portion of the ligated product as opposed to a distinct target-specific portion thereof. Instead, ligated products are separated by gel electrophoresis and detected by autoradiography or fluorescence.

Barany PCR is substantially the same as Wiedmann with the former being relied upon in the outstanding office action as teaching signal to noise ratios, various reporter groups, multiplex formats, detection of multiple mutations, operating conditions, and the use of thermostable ligases. Like Wiedmann, Barany PCR separates ligated products by gel electrophoresis and detects using autoradiography or fluorescence. Using LDR in conjunction with a solid support to capture ligated product sequences by hybridization of an addressable array-specific portion of the product which is distinct from the target-specific portion thereof is nowhere suggested by Barany.

Zaun discloses an apparatus and method for amplifying and detecting target nucleic acids. This procedure involves amplifying with a thermal cycling device and then detecting reaction products on a support having one or more reaction sites. Amplification can be carried out using the polymerase chain reaction ("PCR") or LCR procedures. To capture amplification products, the detection system is provided with a support having a plurality of capture sites to immobilize such products on the support. Zaun discloses capturing amplification products with antibody-antigen binding. The presence of captured nucleic acids is revealed by detection of a label bound to the nucleic acid with a specific binding pair. Such binding is preferably achieved with an antibody-antigen binding pair; however, chemical bonding and complementary polynucleotide procedures are also disclosed.

There is no enabling disclosure in Zaun of utilizing an LDR procedure for detecting single base changes, insertions, deletions, or translocations using oligonucleotide probe sets "configured so that the addressable array-specific portion is comprised of a nucleotide sequence which is distinct from that of the target-specific portions, in order to minimize hybridization between the target-specific portions and the capture oligonucleotides as well as between the target nucleotide sequence and the addressable array-specific portion", as set forth in claims 1 and 138. This feature permits the LDR procedure to be carried out under conditions which facilitate discrimination between target nucleotide sequences and other nucleic acids in a sample without affecting the subsequent detection procedure. See claim 150. Moreover, the sample can be subjected to a single set of ligation detection reaction conditions which facilitates more than one such reaction being carried out at a time. See claim 151. This is the case despite the fact that both the LDR and the solid support capture phases of the present invention involve hybridization to the oligonucleotide probe set. There is no suggestion of this aspect of the present invention in Zaun.

Zaun emphasizes the use of antigen-antibody binding to capture LCR products on a solid support. The problem with such binding is that different antigen-antibody binding pairs have differing levels of affinity, so a tremendous amount of experimentation would be needed to identify a sufficient number of different antigen-antibody binding pairs to capture and distinguish a large number of different LCR products on a solid support. By contrast, capture of ligated products by hybridization of an addressable array-specific portion thereof to a capture probe on a solid support can be carried out by designing different addressable array-specific portions which hybridize to capture probes at similar conditions. The design of such a system is ascertained accurately and easily by matching reaction kinetics, largely by making Tm calculations. It thus can hardly be said that antigen-antibody binding is equivalent, let alone superior, to nucleic acid-nucleic acid hybridization as suggested by Zaun.

There is no mention in Zaun of designing capture oligonucleotide probes and addressable array-specific portions of sufficient length to achieve specificity, yet avoid cross-hybridization. This is because the preferred antibody-antigen binding of Zaun only requires consideration of these binding partners' relative affinity for one another; the concept of cross-hybridization to other binding partners is not considered. The failure to design capture oligonucleotide probes and addressable array-specific portions of proper length can lead to a

false positive product signal being generated on the solid support regardless of whether there is target match or mismatch. Specifically, in the present invention, a match is detected when target-specific portions of the oligonucleotide probes hybridize to a target sequence, undergo ligation, and the ligated product is captured on a solid support by hybridization of an addressable array-specific portion to a capture probe. The second hybridization condition which captures the addressable array-specific portion on the solid support is sufficiently different from the hybridization condition during which ligation took place so that there is no carryover false signal through sandwich hybridization (i.e. hybridization to the capture probe of a first oligonucleotide probe which is bound to the target which is bound to a second oligonucleotide probe, where neither oligonucleotide probe is ligated together). Thus, in the present invention, a mismatch of the oligonucleotide probes when hybridized to a target sequence prevents ligation, and the two oligonucleotide probes dissociate from the target under the second hybridization conditions used to capture the addressable array-specific portion on the solid support.

To the extent Zaun mentions capture with complementary polynucleotides, there is no indication that this involves providing the oligonucleotide probes with an addressable array-specific portion which is distinct from the probes' target-specific portion. It is far more plausible that such polynucleotides instead hybridize to the target-specific portion of the probes. This presents great challenges in designing probes which can undergo both LDR and array hybridization, particularly where multiplexing is involved. The present invention is a major advance over such a format in that the subject oligonucleotide probes can be designed to carry out LDR and solid support capture independently.

The form of LCR utilized by Zaun is gap LCR where the oligonucleotide primers hybridize to a target nucleic acid sequence at spaced apart locations. This leaves a gap between them which is filled by a subsequent polymerase extension step. However, the formation of such a gap makes this procedure ineffective in detecting mutations in adjacent bases, because individual probes for each mutation are likely to produce positive signals if either mutation is present. In addition, when gap LCR is utilized in conjunction with an array capture format, the resulting ligation products would likely be captured at the same location and, therefore, would not be discriminated. For similar reasons, gap LCR would not be able to detect and distinguish slippage events, such as where the wild type sequence has a 10 base poly-A sequence, while the mutant sequence has a 9 base poly-A sequence.

Guo relates to a multiplex PCR amplification procedure. This involves amplifying and detecting target nucleic acids using fluorescently labeled tags where the amplification products are denatured to render them single stranded, captured on a solid support with oligonucleotide probes, and detected. An important distinguishing feature of the present invention is that there is no need to denature ligated products to single stranded nucleic acid products; such products are either already single stranded or can be detected even if the ligated product is still hybridized to its corresponding target nucleic acid. Guo does not suggest using LDR at all, let alone in conjunction with a solid support to capture ligated product sequences resulting from LDR by hybridization to a capture oligonucleotide. In fact, Guo teaches away from the present invention by teaching that genetic polymorphisms can be detected by use of allele-specific oligonucleotides immobilized on glass slides.

Reddy relates to a composition and method for detection of analytes by using an assay system where one component is attached to a first member of a pair of complementary sequences forming a double stranded nucleic acid. The assay is carried out in the presence of a support to which the complementary nucleic acid is attached. Upon hybridization, the complementary nucleic acids hybridize to one another and are immobilized on the solid support. Immobilized analytes (bound to their respective antibodies) are then selectively displaced from the solid support, using a displacer oligonucleotide, with the analyte's presence in solution then measured by detection of labeled analyte. In particular, the portion of oligonucleotide sequence which hybridizes to the solid support has a length and Tm which gives the displacer oligonucleotide sufficient opportunity to displace the antibody-analyte complex from the solid support. In fact, were one to adapt the procedure of Reddy to the design of the addressable array-specific portion and the capture probes of the present invention, these oligonucleotides would only hybridize under conditions permitting false positive signal due to sandwich hybridization. This occurs because short complementary sequences are needed to achieve Reddy's displacement technique. Thus, Reddy teaches away from the present invention. Further, Reddy has nothing to do with LDR or the use of a solid support to capture ligated product sequences resulting from LDR by hybridization to a capture oligonucleotide. His technique is used to carry out immunochemical assays where the assay involves antibody-antigen or protein-protein binding where the target itself is labeled. In the present invention, the presence of product is amplified and the existence of target is determined by detection of labeled ligation product as opposed to target itself. Even

if one of ordinary skill in the art were to adapt Reddy's technique to LDR (which there is absolutely no suggestion of), he would configure the nucleic acid attached to the solid support to be complementary to the target specific portion of the ligated product; no addressable array-specific portion would be needed. As noted above with respect to Zaun, the present invention constitutes a major advance over such an arrangement.

As noted above, Weidmann, Barany PCR, and Zaun all relate to procedures involving LDR or LCR. On other hand, neither Guo nor Reddy involve LDR or LCR in any way whatsoever. Accordingly, one of ordinary skill in the art would have had no motivation, let alone ability, to adapt the teachings of Guo and Reddy to the LDR procedures of Weidmann, Barany PCR, and Zaun.

Even if the cited references were combinable, which they are not, their combination would not teach the claimed invention. As noted above, neither Weidmann, Barany PCR, Zaun, Guo, nor Reddy suggest adapting an LDR procedure to a subsequent solid phase capture procedure, as claimed. Claims 1 and 138 require oligonucleotide probe sets "configured so that the addressable array-specific portion is comprised of a nucleotide sequence which is distinct from that of the target-specific portions, in order to minimize hybridization between the target-specific portions and the capture oligonucleotides as well as between the target nucleotide sequence and the addressable array-specific portion". Although Zaun involves LCR and solid support capture, there is no suggestion of this feature. Zaun, at best, only suggests a solid support where solid capture involves hybridization to the target-specific portion of ligation products. This facilitates the procedure of the present invention where "sequences differing by one or more single-base changes, insertions, deletions, or translocations are discriminated from one another during the ligase detection reaction and the discriminated sequences are detected as a result of capture on the solid support" as set forth in claim 150. It also facilitates use of a "single set of ligase detection reaction conditions" pursuant to claim 151. Again, the combination of Weidmann, Barany PCR, Zaun, Guo, and Reddy provide no suggestion of any of these aspects of the present invention. Accordingly, the rejection based upon this combination of references should be withdrawn.

It is further submitted that this combination of references also fails to teach the parenthetical features of the following dependent claims: 4 (mismatch at a base adjacent to a base at the ligation junction), 11 (quantifying target nucleotide sequences), 12, and 14-34 (detecting multiple allele differences), and 13 (target specific portions of oligonucleotide

probes with substantially the same melting temperatures). Nowhere in the outstanding office action is it explained where these features, as specifically claimed by applicants, are taught by the cited references. Although there is some reference to Guo for some (but not all) of these features, Guo, in any event, does not disclose carrying out these procedures in the manner claimed by applicants which involves the use of LDR. As to claims 4 and 13, the requisite art or examiner's declaration needed to establish the PTO's position that the use of mismatch at a base adjacent to the junction and similar melting temperatures, respectively, would have been *prima facie* obvious has not been provided. Therefore, the rejection of claims 4 and 11-34 based on the combination of Weidmann, Barany PCR, Zaun, Guo, and Reddy should be withdrawn.

In the outstanding office action, great reliance is placed on Reddy's teaching of carrying out an assay by immobilizing an oligonucleotide on a solid support by hybridization instead of assaying for an analyte with an immobilized antibody specific to that analyte. How this provides any motivation to carry out an LDR process in conjunction with capture on a solid support is not explained. The mere fact that the cited references can be generally characterized as labeled products does not in any way dispense with the U.S. Patent and Trademark Office's ("PTO") obligation to articulate why one of ordinary skill in the art would combine them in the specific manner proposed in the outstanding office action. Moreover, the outstanding office action makes no attempt to respond to applicants' arguments (reiterated above) that even if the references were combinable, which they are not, that combination would not teach the claimed invention. Again, as set forth *supra.*, if one of ordinary skill in the art had any reason to capture LDR products. Zaun, Guo, and Reddy would capture such products at the same locations where they underwent target-specific hybridization during the LDR phase of the process. There is no indication in the outstanding office action of where the art of record suggests utilizing oligonucleotide probe sets "configured so that the addressable array-specific portion is comprised of a nucleotide sequence which is distinct from that of the target-specific portions, in order to minimize hybridization between the target-specific portions and the capture oligonucleotides as well as between the target nucleotide sequence and the addressable array-specific portion," as set forth in claims 1 and 138. There is no attempt to explain how this art teaches that "sequences differing by one or more single-base changes, insertions, deletions, or translocations are discriminated from one another during the ligase detection reaction and the discriminated

sequences are detected as a result of capture on the solid support" as set forth in claim 150 or the use of a "single set of ligase detection reaction conditions," pursuant to claim 151. Thus, the outstanding office action provides no rational basis for one of ordinary skill in the art to combine the 5 cited references (involving very distinct procedures), nor any explanation of how the specific features of the claims with which applicants made it possible to effectively combine the LDR and solid support capture formats are taught by these references. In view of the PTO's failure to carry its burden of making a *prima facie* case of obviousness, the rejection based upon the combination of Wiedmann, Barany PCR, Zaun, Guo, and Reddy must be withdrawn.

The rejection of claims 6-10, 22, and 23 under 35 U.S.C. § 103 for obviousness over Wiedmann in view of Barany PCR, Zaun, Guo, Reddy, and Telenti, et. al., "Competitive Polymerase Chain Reaction Using an Internal Standard: Application to the Quantitation of Viral DNA," J. Virol. Methods 39: 259-68 (1992)("Telenti") is respectfully traversed.

Telenti is cited as teaching that PCR can be quantitated by providing a known amount of an internal standard. However, this reference does not disclose the use of an internal standard in conjunction with an LDR process, nor does it involve the use of arrays. Therefore, Telenti does not overcome the above-noted deficiencies of Wiedmann, Barany PCR, Zaun, Guo, and Reddy.

The rejection of claims 78, 82, and 84-86 under 35 U.S.C. § 103 for obviousness over Wiedmann in view of Barany PCR, Zaun, Guo, Reddy, and Sambrook, et. al., Molecular Cloning (1989)("Sambrook") is respectfully traversed.

Sambrook is cited for its teachings regarding hybridizing to immobilized nucleotides, barrier oligonucleotides, exonucleases to destroy nucleotides, and stripping blots. However, Sambrook does not involve the use of LDR in conjunction with arrays. In view of these deficiences, the rejection based on the combination of Wiedmann, Barany PCR, Zaun, Guo, Reddy, and Sambrook should be withdrawn.

The rejection of claims 1-43, 45-66, 75-80, 82-88, and 138-149 under 35 U.S.C. § 112 (2nd para.) is respectfully traversed. Applicants submit that the term "minimize" in the phrase "in order to minimize hybridization between the target-specific portions and the capture oligonucleotides as well as between the target nucleotide sequence[(s)] and the addressable array-specific portion" is fully understandable to one of ordinary skill in the art.

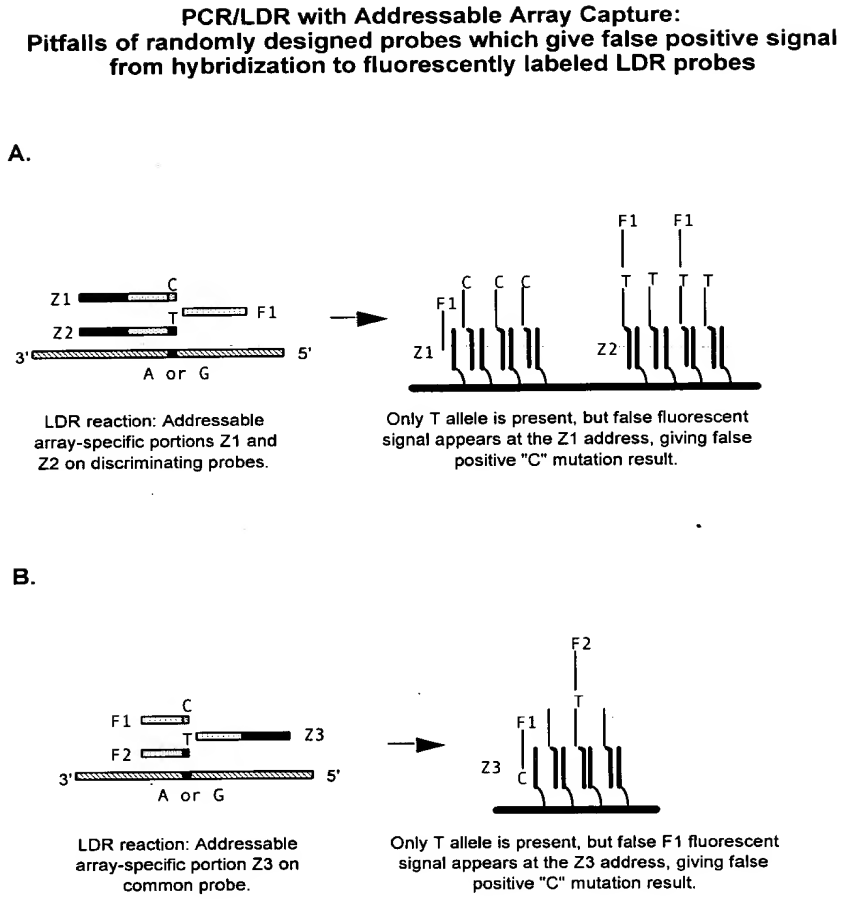
The dictionary definition of "minimize" is well known to such skilled artisans as meaning "to make as small as possible". Webster's American Dictionary (Smithmark Publishers 1999). From this, it would have been readily understood that the claims call for configuring the oligonucleotide probes so that hybridization of the target-specific portions to the capture oligonucleotides as well as of the target nucleotide sequence and the addressable array-specific portion are kept small. The precise extent to which such interactions are reduced is neither necessary to practice the present invention nor to understand what the claims cover. Accordingly, the rejection of the claims under 35 U.S.C. § 112 (2nd para.) should be withdrawn.

The rejection of claims 1-5, 11-21, 24-43, 45-66, 75-77, 79-80, 83, 87-88, and 138-151 under 35 U.S.C. § 103 for obviousness over Weidmann, Barany PCR, Guo, and either U.S. Patent No. 5,981,176 to Wallace or WO 93/25563 to Wallace (collectively "Wallace") or U.S. Patent No. 5,391,480 to Davis et. al., or WO 90/11272 to Davis, et. al., (collectively "Davis") is respectfully traversed.

As described in the attached Declaration of Francis Barany under 37 C.F.R. § 1.132 ("Barany Declaration"), ¶4, the present application relates to a procedure of identifying one or more of a plurality of sequences differing by one or more single-base changes, insertions, deletions, or translocations in a plurality of target nucleotide sequences by using the combination of ligase detection reaction ("LDR") and array capture with oligonucleotides on a solid support.

In this process, the capture oligonucleotide probe needs to be carefully designed for capture of an LDR ligation product to avoid false positive or false negative signals which would result from the random design used with the primer extension technique described below (Barany Declaration ¶6). A random design may be used on the primer extension approach, because the fluorescent label is on the dideoxynucleotide and, therefore, would not hybridize to any address on the array unless incorporated onto a (presumably correctly hybridized) probe (Id.). In contrast, with LDR, the fluorescent label(s) is (are) either on the discriminating probes or on the common probe (Id.). Incorrect hybridization of one of these to an address on the array would give a false positive signal, with potentially disastrous consequences (Id.). This is illustrated in Figure 2 (Id.).

Figure 2

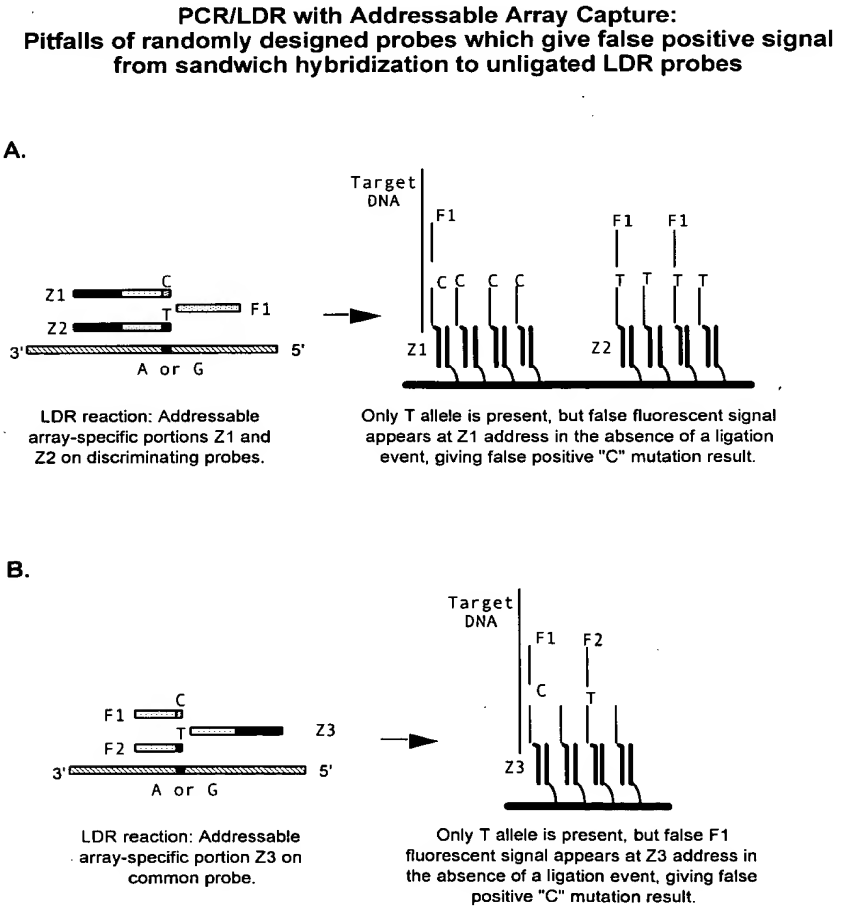


In Figure 2A, the common probe can hybridize somewhat to the Z1 address on the array, giving a false positive signal for the "C' allele (Id.). In Figure 2B, the allele specific probe containing the F1 fluorescent group can hybridize somewhat to the Z3 address on the array, giving a false positive signal for the "C' allele (Id.). In a typical LDR reaction, 10% to 20% of the LDR probes are converted to product (Id.). However, PCR/LDR is very sensitive and can distinguish a single point mutation in a thousand-fold excess of wild-type DNA (Id.). If the technique is being used to detect a low abundance mutation, the percent of product generated ranges from 0.01% to 1% of the starting LDR probes (Id.). That rare product then competes with unreacted addressable array specific portions for hybridization to the correct address (Id.). Even low levels of false hybridization of the fluorescently labeled LDR probe

to that or another address may give a false positive result (Id.). In a multiplex system, none of the fluorescently labeled LDR probes should falsely hybridize with any of the addresses on the array (Id.). Thus, considerable care is required to properly design the capture oligonucleotide probes to avoid false positive signals (Id.).

Another problem with random design of the capture oligonucleotide is the potential for sandwich hybridization, because the capture oligonucleotide probe's Tm was too low (Barany Declaration ¶7). For the probes of the present application, stable addressable array-specific portions must be formed at a temperature which is sufficiently above the ligation reaction temperature (65°C) (Id.). Unless this is achieved, false positive signals may result from hybridization between adjacent unreacted LDR probes which are hybridized to the target (Id.). This is illustrated in Figure 3 (Id.).

Figure 3



In Figure 3A, a sandwich hybridization of unligated common probe and captured "C" allele addressable array-specific portion containing a probe with target DNA giving a false positive signal for the "C' allele (Id.). In Figure 3B, a sandwich hybridization of unligated allele "C" probe and the captured probe containing a common addressable array-specific portion with target DNA gives a false positive signal for the "C' allele (Id.). The addressable array-specific portion design of the present invention provides calculated T_m values to avoid this problem (Id.).

Thus, it is important to design the addressable array-specific portion of each LDR probe and the complementary capture oligonucleotides such that the addressable array-specific portions of each oligonucleotide probe hybridizes to its complementary sequences on the addressable array under hybridization conditions where either direct or sandwich (indirect) hybridization of any labeled LDR probe or LDR product to a non-cognate address is minimized or below the limit of detection (Barany Declaration ¶8).

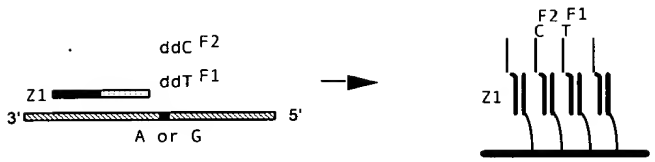
Wallace relates to a method of detecting and discriminating between nucleic acid sequences (Barany Declaration ¶ 10). The method utilizes a primer having a 3' portion which is specific for the desired nucleic acid sequence and a 5' portion which is complementary to a preselected nucleic acid sequence (Id.). Extension of the 3' portion of the primer with a labeleled deoxynucleoside triphosphate yields a labeled extension product if, but only if, the template includes the target sequence (Id.). The labeled extension product is detected by hybridization of the 5' portion to the preselected sequence with the preselected sequence preferably being bound to a solid support along with many other sequences (Id.).

Figure 4 summarizes the single nucleotide primer extension technique ("SNUPE") using a randomized capture portion, essentially as described by Wallace; however, Wallace only utilizes this technique with radioactively labeled dideoxynucleotides.

Figure 4

PCR/SNUPE with Addressable Array Capture

A.



SNUPE reaction: Addressable array-specific portion Z1 on the primer. Extend with polymerase and dideoxynucleotides ddC and ddT which have fluorescent labels F2 and F1.

Heterozygous: C and T alleles.

(Barany Declaration ¶ 11). In this scheme, a primer specific to the gene and containing a random 20 mer sequence on the 5' end of a target specific portion, hybridizes to the target directly adjacent to the base in question (Id.). A non-proofreading polymerase extends the primer with a labeled dideoxynucleotide (in this case, fluorescently labeled F1 or F2) (Id.). Capture of the extended primer by hybridization at the appropriate address on an array (Z1) and determination of the fluorescent label at that address allows the different alleles to be distinguished (Id.).

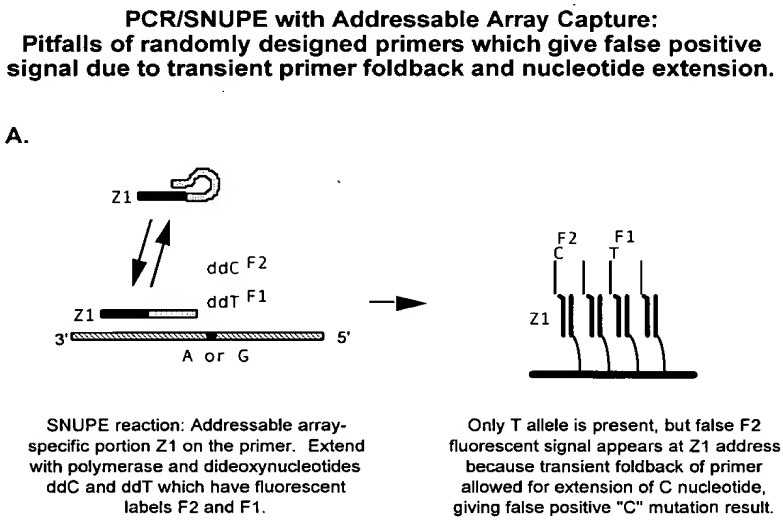
A number of the aspects of SNUPE make its features inapplicable to detecting single based differences by the ligase detection reaction ("LDR")/universal array capture technique of the present invention (Barany Declaration ¶ 12). In general, SNUPE is not adaptable to LDR/universal array capture, because the former uses random primers and achieves target discrimination as a result of polymerase extension while the latter uses an additional probe to distinguish target sequences (Id.). The use of polymerase extension SNUPE creates a number of opportunities to generate false positive results which would not happen with LDR/universal array capture (Id.). In view of this deficiency in SNUPE, one distinguishing nucleic acids with LDR/universal array capture based on single base differences would regard the SNUPE technique as relevant (Id.).

Firstly, Wallace's process is limited to using the random capture probe on the 5' side (Barany Declaration ¶ 13). Thus, all the allele signals must come to the same address on the array (Id.). Since different fluorophores may overlap with one another, this leads to

significant problems when attempting to distinguish multiple potential mutations in a single codon, for example the K-ras G12 codon (Id.). While it may be possible to leave out the dideoxynucleotide which would be inserted opposite wild-type DNA, this would work for only one position at a time (Id.). Thus, the technique of Wallace is not amenable to multiplex detection of multiple mutations at multiple sites (Id.). One cannot place the capture oligonucleotide on the 3' side since polymerases only synthesize in the 5'-3' direction (Id.).

Secondly, in the process of Wallace, the gene specific primer may form a transient hairpin and be extended by one base which is complementary to its own sequence of the random probe (Barany Declaration ¶14).

Figure 5

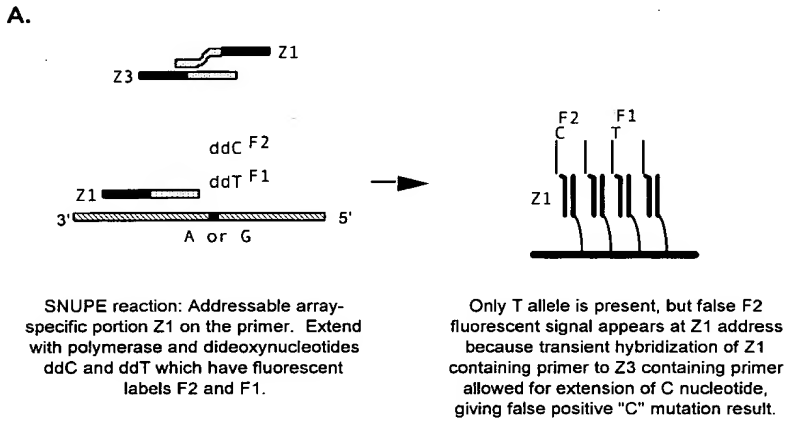


This is shown in Figure 5 where the addressable array-specific portion Z1 of the primer, instead of hybridizing to the target nucleic acid, folds-back, hybridizes to itself, and is extended by polymerase (Id.). As a result, if only the nucleic acid with the T allele is present in the sample, the target-specific extension of the primer yields an extension product with the F1 label, while the primer-specific extension may happen to yield an extension product with the F2 label (Id.). When captured on the solid support, both of these products are displayed on the support even though no target having the C allele is present in the sample (Id.).

Thirdly, the process of Wallace is also susceptible to transient hybridization of 2 primers followed by polymerization (Barany Declaration ¶15).

Figure 6

**PCR/SNUPE with Addressable Array Capture:
Pitfalls of randomly designed primers which give false positive signal due to transient two primer hybridization and nucleotide extension.**



As shown in Figure 6, the primer with an addressable array-specific portion Z1 is intended to be useful in detecting T or C alleles in the target nucleic acid (Id.). However, in cases of multiplex detection, where a different primer having an addressable array-specific portion Z3, is also present to detect a different allele in another target sequence, those primers may hybridize to another and be extended by a labeled dideoxynucleotide other than what they would otherwise be extended with if hybridized to the target nucleic acid sequence (Id.). As shown in Figure 6, if only the nucleic acid with the T allele is present in the sample, the target-specific extension of the primer yields an extension product with the F1 label, while the primer-specific extension may happen to yield an extension product with the F2 label (Id.). When captured on the solid support, both of these products are displayed on the support even though no target having the C allele is present in the sample (Id.).

The susceptibility of Wallace to false positive detection due to its use of randomly designed capture probes (where the sequence numbers are set forth in Wallace) is further demonstrated by the teachings of Wallace as follows (Barany Declaration ¶ 16):

A. Random capture sequences which form hairpins to themselves (Id.).

TCAGTTACCACGGTAACTGG This random sequence has an 8 base inverted repeat, forms hairpin, and will not hybridize well to the capture oligonucleotide probe on an addressable array.

B. Random capture oligonucleotide probe sequences which hybridize to each other (Id.).

CATAATGCGGTCTCGATACG Seq ID # 8

CAAATGCGGTCTCGATATCG This slight modification of Seq. ID # 8 has an 8 base repeat on the end and will hybridize to another molecule during the coupling reaction and, therefore,will not hybridize to an addressable array-specific portion.

CAAATGCGGTCTCGATATCG
GCTATAGCTCTGGCGTAAAC

C. Random capture sequences which are similar, allowing for hybridization and capture at the wrong address (Id.).

ATGGGCTCCTGCGTAAATCA Seq ID # 2
AGTGCGCTCTCTTGAGCAAA Seq ID # 3

ATGCGCTCTCCTGGGTAAAA Modified Seq ID # 17,
AGTGCGCTCTCTTGAGCAAA Seq ID # 18. Slight modification shows a high degree of similarity between oligonucleotides (16/20) allowing for false hybridization at incorrect addresses.

D. Random capture sequences which hybridize with any other LDR probe in the LDR mixture, thus precluding correct hybridization on the array (false-negative) or allowing for sandwich or indirect hybridization to the array resulting in false-positive signal (Id.).

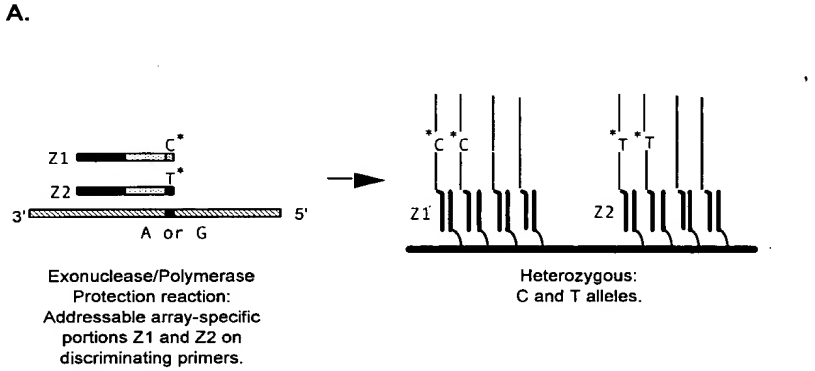
As shown above, the SNUPE process is susceptible to detection of false positive results due to its efforts to discriminate target nucleic acid sequences by use of random primers and polymerase extension (Barany Declaration ¶ 17). This problem is worsened when the SNUPE process is preceded by PCR, because, in that case, all dNTPs from that earlier reaction must be destroyed to prevent SNUPE primer extension before an incorrect dideoxynucleotide is inserted at a downstream base (Id.). In addition, SNUPE

cannot detect small insertions, deletions, or slippage events in mono- or di-nucleotide repeats (Id.). By contrast, the LDR/universal array capture technique is able to detect such conditions with minimal risk of false signals by careful design of both the oligonucleotide probes used for LDR and the oligonucleotide probes used for array capture (Id.). As a result, the features of the SNUPE process would have little applicability to the LDR/universal array capture technique (Id.).

U.S. Patent No. 5,391,480 and WO 90/11272 both to Davis, et al. (collectively, "Davis") discloses a method of detecting a nucleotide at a specific location within a nucleic acid using exonuclease activity (Barany Declaration ¶ 18).

Figure 7

Exonuclease/Polymerase Protection with Addressable Array Capture



In particular, as shown in Figure 7 of Davis, a test sample of DNA is treated with a labelled oligonucleotide having a primer portion capable of hybridizing to target DNA, if present in the test sample (Id.). The label is attached to a nucleotide in the primer portion at a test position and that nucleotide will hybridize to the target DNA if there is complementarity at that position (Id.). On the other hand, if such complementarity is lacking, the labelled nucleotide will not be bound (Id.). As a result, a subsequent exonucleolytic treatment step, which follows a polymerase extension procedure, will excise the label if complementarity is absent at the test position (Id.). The primer includes a tail portion (Z1 and Z2 in Figure 7) so that the primer can be immobilized on a solid support and detected when the primer is

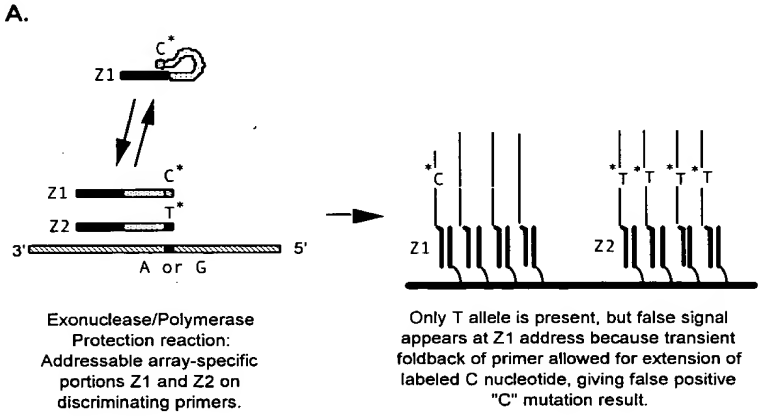
contacted with the solid support under conditions effective to hybridize the tail portion to a complementary oligonucleotide on the solid support (Id.). In Figure 7, since the sample is heterozygous for target nucleic acids with the C and T alleles, labeled primers are captured at both the Z1 and Z2 addresses (Id.).

A number of aspects of the Davis process make its features inapplicable to detecting single base differences by LDR/universal array capture (Barany Declaration ¶ 19). The Davis process is not adaptable to LDR/universal array capture, because the former is susceptible to false positive detection as a result of its use of hybridization, exonucleolytic treatment, and polymerase extension (Id.). Since these opportunities for the Davis process to detect false positives are not present in LDR/universal array capture, the Davis process would not be viewed as relevant to LDR/universal array capture (Id.).

Firstly, in the process of Davis, the gene specific primer may form a transient hairpin and be extended by several bases which are complementary to its own sequence and/or the random probe sequence (Barany Declaration ¶ 20).

Figure 8

Exonuclease/Polymerase Protection with Addressable Array Capture: Pitfalls of randomly designed primers which give false positive signal due to transient primer foldback and nucleotide extension.



Exonuclease/Polymerase Protection reaction: Addressable array-specific portions Z1 and Z2 on discriminating primers.

Only T allele is present, but false signal appears at Z1 address because transient foldback of primer allowed for extension of labeled C nucleotide, giving false positive "C" mutation result.

This is shown in Figure 8 where the addressable array-specific portion Z1 of the primer, instead of hybridizing to the target nucleic acid, folds-back, hybridizes to itself, and is extended by polymerase (Id.). As a result, if only the nucleic acid with the T allele is present

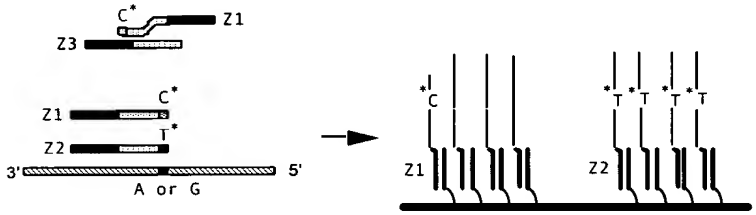
in the sample, the target-specific extension of the primer yields an extension product with a label at the Z2 address, while the primer-specific extension may happen to yield an extension product with a label at the Z1 address (Id.). When captured on the solid support, both of these products are displayed on the support even though no target having the C allele is present in the sample (Id.).

Secondly, the process of Davis is also susceptible to transient hybridization of 2 primers followed by polymerization (Barany Declaration ¶ 21).

Figure 9

Exonuclease/Polymerase Protection with Addressable Array Capture: Pitfalls of randomly designed primers which give false positive signal due to transient two primer hybridization and nucleotide extension.

A.



Exonuclease/Polymerase Protection reaction: Addressable array-specific portions Z1 and Z2 on discriminating primers.

Only T allele is present, but false signal appears at Z1 address because transient hybridization of Z1 containing primer to Z3 containing primer allowed for extension of labeled C nucleotide, giving false positive "C" mutation result.

As shown in Figure 9, the primer with an addressable array-specific portion Z2 is intended to be useful in detecting the T allele in the target nucleic acid (Id.). However, in cases of multiplex detection, where a different primer having an addressable array-specific portion Z2, is also present to detect nucleic acids with the C allele, those primers may hybridize to another and be extended by polymerase so that a label which should have been removed by exonucleolytic treatment remains in place (Id.). As shown in Figure 9, if only the nucleic acid with the T allele is present in the sample, the target-specific extension of the primer with the Z2 portion yields a labelled extension product, while the primer-specific extension may happen to yield an extension product with a labelled extension product which is produced

when target nucleic acid with the C allele (which is absent here from the sample being analyzed) is present (Id.). When captured on the solid support, both of these products are displayed on the support even though no target having the C allele is present in the sample (Id.).

As shown above, the Davis process is susceptible to detection of false positive results due to it efforts to discriminate target nucleic acid sequences by use of primers suitable for hybridization, exonucleolytic primers, and polymerase extension (Barany Declaration ¶ 22). The Davis process starts with labeled probe which is subsequently destroyed by exonuclease digestion (Id.). Thus, if exonuclease digestion is incomplete, then the low level of false positive signal would be mistakenly interpreted as indicating the presence of low level mutant target (Id.). In addition, the Davis process cannot detect small insertions, deletions, or slippage events in mono- or di-nucleotide repeats (Id.). By contrast, the LDR/universal array capture technique is able to detect such conditions, including low levels of mutant target, with minimal risk of false signals by careful design of both the oligonucleotide probes used for LDR and the oligonucleotide probes used for array capture (Id.). As a result, the features of the Davis process would have little applicability to the LDR/universal array capture technique (Id.).

For all of these reasons, the rejection based on the combination of Weidmann, Barany PCR, Guo, and either Wallace or Davis should be withdrawn.

The rejection of claims 1-43, 45-66, 75-77, 79-80, 83, 87-88, and 138-151 under 35 U.S.C. § 103 for obviousness over Wiedmann in view of Barany PCR, Guo, either Wallace or Davis, and Telenti or U.S. Patent No. 5,667,974 to Birkenmeyer, et. al., ("Birkenmeyer") is respectfully traversed.

The combination of Wiedmann, Barany PCR, Guo, either Wallace or Davis, and Telenti cannot be properly used to reject the claims of the present application for substantially the reasons noted *supra*.

Birkenmeyer is cited as teaching the use of an internal standard nucleic acid for the quantification of ligase-mediated amplification reaction products. Birkenmeyer is substantially the same as Zaun in that Birkenmeyer discloses a process for quantitatively detecting the amount of a target nucleic acid sequence in a test sample by subjecting the test sample to an amplification reaction followed by detection with capture probes immobilized on a solid phase. Thus, the above comments relating to Zaun are equally applicable to

Birkenmeyer in distinguishing it from the present invention. Therefore, the combination of Wiedmann, Barany PCR, Guo, Reddy, either Wallace or Davis, and Birkenmeyer cannot be properly used to reject the claims.

The rejection of claims 1-5, 11-21, 24-43, 45-66, 75-80, 82-88, and 138-151 under 35 U.S.C. § 103 for obviousness over Wiedmann in view of Barany PCR, Guo, either Wallace or Davis, and Sambrook is respectfully traversed for substantially the reasons noted *supra*.

In view of all the foregoing, it is submitted that the present application is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: January 25, 2001

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1/25/01
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Wendy L. Harrold